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A REVIEW OF ANAPHYLAXIS, WITH ESPECIAL REFERENCE TO IMMUNITY.*†

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ANAPHYLAXIS (ἄνα, against, and φυλός, guard, or φυλάξις, protection), also called hypersusceptibility, supersensitiveness, is a condition of unusual or exaggerated susceptibility of the organism to foreign substances. The word *ana*phylaxis suggests the contrary condition to *pro*phylaxis; it may be congenital or acquired; it is specific in nature. The condition of anaphylaxis may be brought about by the introduction of any strange proteid into the body. Hypersusceptibility to proteins that are non-poisonous in themselves may readily be induced in certain animals.

An animal may be in a condition of hypersusceptibility and immunity at the same time. The two conditions are closely interwoven. The one may be dependent upon the other. von Pirquet advises that the term "immunity" be limited to indicate the condition of complete resistance in which no clinical reaction occurs, when poisons (such as diphtheria, tetanus, etc.) are introduced into the organism. He suggests the term "allergie" to indicate conditions of acquired immunity associated with anaphylaxis, such as that induced by vaccinia against variola, that of the luetic against syphilis, or that produced by one attack of some of the acute specific infections. This condition of allergie manifests itself in the renewal of the infection in an entirely different manner from the reaction to the primary infection.

The tuberculin and mallein reactions are well-known instances of anaphylaxis. These substances are not poisonous when introduced into a healthy individual, but the tuberculous individual is anaphylactic to tuberculin, and an individual suffering with glanders is in a state of hypersusceptibility to mallein.

The best-studied instance of anaphylaxis is that produced in the guinea-pig by the injection of a foreign proteid, for example, horse

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serum, egg white, milk, etc. Especial study has been made of the anaphylactic action of the blood serum of the horse, partly because that serum is so much used in serum therapy.

It has long been known that the blood of certain animals is poisonous when transfused or injected into certain other species. Many instances might be cited showing that the blood serum of an animal of one species has poisonous properties when injected into an animal of another species. But the blood serum of the horse apparently lacks such poisonous action. Very large quantities of the blood serum of the horse may be injected into man, rabbits, guinea-pigs, and many other animals without serious inconvenience, except occasionally a slight reaction at the site of inoculation.

In a certain proportion of cases the injection of horse serum into man is followed by urticarial eruptions, joint pains, fever, swelling of the lymph nodes, edema, and albuminuria. This reaction, which appears after an incubation period of 8 to 13 days, has been termed by von Pirquet and Schick the "serum disease."

In exceptional instances sudden death has followed an injection of horse serum in man.

We have shown that ordinarily horse serum is a comparatively bland and harmless substance when injected into certain animals; but these animals may be rendered so susceptible that an injection of horse serum may produce death or severe symptoms. For example, large quantities of horse serum may be injected subcutaneously into the peritoneal cavity, into the brain, or directly into the circulation of a guinea-pig without apparently causing the animal the least inconvenience. However, if a guinea-pig is injected with a small quantity, say 1/250 c.c., of horse serum and after the expiration of a certain interval is again injected with horse serum the result will probably be fatal. The first injection of horse serum has sensitized the animal in such a way as to render it very susceptible to a second injection of horse serum.

A certain time must elapse between the first and second injections before this toxic action is able to manifest itself. This "period of incubation" is from 10 to 12 days, and corresponds suggestively with the period of incubation of the serum disease which von Pirquet and Schick place at 8 to 13 days.

Guinea-pigs may be sensitized with exceedingly small quantities of horse serum. In most of our work we used quantities less than 1/250 c.c. and we found in one instance that 1/1,000,000 c.c. of horse serum was sufficient to render a guinea-pig susceptible.

It also requires very small quantities of horse serum, when given in a second injection, to produce poisonous symptoms. One-tenth c.c. injected into the peritoneal cavity is sufficiently to produce serious symptoms.

While at first we thought that diphtheria antitoxin had some relation to this action, we are now able to state positively that it has nothing whatever to do with the poisonous effects of horse serum; further, that diphtheria antitoxin in itself is absolutely harmless. The toxic action which we have studied is caused by a principle in normal horse serum and is entirely independent of the antitoxic properties of the serum.

Historical.—The fact that guinea-pigs which had been used for the testing of diphtheria antitoxin frequently died when later given an injection of serum has been known almost since the discovery of diptheria antitoxin, but no one seems to have attached any significance to the relation between the two injections until the fall of 1905. Most of the workers with serum regarded it as an accident pure and simple or considered that the animal's vital resistance had been lowered by the first treatment; some even thought that it was the effect of cold, as the sera were usually kept in the ice chest and were injected at once after removing from the ice box. Theobald Smith, however, evidently had the problem in mind as he spoke to Ehrlich about it when the latter was in this country in 1904, the result being Otto's work. When we first encountered the phenomenon it at once occurred to us that there might be some relation between it and sudden death after the injection of antitoxin in man, and our work was begun in the hope of throwing light on this unfortunate accident. The results of our work on this most interesting subject have been published in a number of papers[1] since April, 1906. We foresaw early in our work that the phenomenon very probably had a close relation to immunity and our recent work with proteids obtained from the bacterial cells has shown this to be true. We have been able to produce anaphylaxis in guinea-pigs with bacterial extracts and have found that these animals possess an immunity to the corresponding infections.

Early in the last century Magendie[2] (1839) found that rabbits which had tolerated two intravenous injections of egg albumin without any ill effects immediately succumbed to a further injection made after a number of days. Later, workers with precipitin have frequently found that some of their animals died suddenly during the course of treatment from no apparent cause, while what really happened was they were in a state of anaphylaxis to the foreign proteid. Other analogous instances may be found scattered throughout the early literature.

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[1] M. J. Rosenau and John F. Anderson, *Bull. No. 29*, Hyg. Lab., U. S. Pub. Health and Mar.-Hosp. Serv., Washington, 1906; *Jour. Med. Res.*, 1906, 10, p. 179; *Jour. Amer. Med. Assn.*, 1906, 47, p. 1007. See also John F. Anderson, *Bull. No. 30*, Hyg. Lab., U. S. Pub. Health and Mar.-Hosp. Serv., Washington, 1906; *Jour. Med. Res.*, 1906, 10, p. 241; *Ibid.*, p. 259.

M. J. Rosenau and John F. Anderson, *Bull. No. 36*, Hyg. Lab., U. S. Pub. Health and Mar.-Hosp. Serv., Washington, 1907; *Jour. Med. Res.*, 1907, 11, p. 381; *Jour. Infect. Dis.*, 1907, 4, p. 552.

[2] German translation by Krupp, Leipzig, 1839; cited by Ehrlich, *Col. Stud. Imm.*, p. 332.

Knorr[1] (1895) found that guinea-pigs develop an increasing sensitiveness to tetanus toxin.

Hericourt and Richet[2] (1898) in studying the effects of eel serum on dogs found that they were not able to immunize them against the serum, but that on the contrary there was an increasing sensibility to it so that finally the dogs died.

Behring and Kitashima[3] (1901) repeating some of Knorr's work, also found an increasing sensitiveness to tetanus toxin on the part of guinea-pigs.

Portier and Richet[4] (1902) found that if dogs were given a very small dose of a glycerin extract from the tentacles of actinia, and then in 15 or 20 days given a second small dose, the animals quickly succumbed. The dose given was so small as to cause no symptoms in a normal animal. They appear to be the first to use the word "anaphylaxis" to indicate hypersensitiveness to a poison.

Arthus[5] (1903) studied the effect of repeated injections of horse serum on rabbits and found that if the rabbit be given repeated injections of horse serum at some days interval it caused, even in small doses, results which, according to the number of the previous injections and methods of injection, were local or general and benign or grave.

Richet[6] (1904-5) in studying two poisons (congestin and thalassin) extracted from actinia found that if a very small dose, which caused practically no symptoms in a dog, was followed after 22 days by another small dose the animal become very sick or quickly died.

Pirquet and Schick[7] in 1905, in a monograph upon the serum disease described in detail this syndrome which sometimes follows injections of horse serum in man. They show that the symptoms of this "disease" when caused by a second injection may either appear at once (the immediate reaction) or after a shortened period of incubation (the accelerated reaction). From these clinical observations Pirquet and Schick draw original and far-reaching conclusions. They show the relation of these clinical observations to the phenomenon of hypersusceptibility, and indicate the importance of these facts in general pathology. They draw attention to the analogy to the tuberculin reaction as a well-known instance of hypersusceptibility. Pirquet and Schick believe that the serum reactions give a possible explanation of the period of incubation of infectious diseases and finally conclude that the immunity caused by vaccinia and a group of infections is due to the power of immediate reaction acquired by the organism.

During Ehrlich's visit to this country in 1904, Theobald Smith told him that guinea-pigs which had been used in testing the potency of diphtheria antitoxin become acutely sick or die if injected subcutaneously several weeks later with several cubic centimeters of normal horse serum. Ehrlich gave the problem to Otto, who worked out many of the essential features of the phenomenon. This article,[1] published late in 1905, came to our attention while we were correcting the page proofs of our first paper upon the same subject. It seems that the work was done about the same time, but Otto's paper appeared first. Otto showed what is now well known to be the result of a second injection of horse serum into guinea-pigs. He demonstrated that the diphtheria poisons play no part in the phenomenon. However he found that guinea-pigs first treated with mixtures of diphtheria toxin and serum are more susceptible than those treated with serum alone. Otto showed further that immunity to the poisonous action of the serum injection may be acquired by repeated injections of large amounts of serum at short intervals. He demonstrated that this hypersusceptibility bears no relation to the specific precipitins. Finally Otto discusses the relation of the Theobald Smith phenomenon to the cases of reinjection in man and cites instances of alarming symptoms following the second injection of antitoxic horse serum.

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[1] *Habilitätionsschrift*, 31 pp., 8 pls., 8 vo; Marbourg, 1895.

[2] *Compt. rend. soc. biol.*, 1898, 53, p. 137.

[3] *Berl. klin. Wchnschr*, 1901, 38, p. 157.

[4] *Compt. rend. soc. biol.*, 1902, 54, p. 170.

[5] *Compt. rend. soc. biol.*, 1903, 55, p. 817.

[6] Arch. di fisiol., 1904, 1, p. 129. *Compt. rend. soc. biol.*, 1905, 58, p. 109. *Ibid.*, p. 112.

[7] Dr. C. von Pirquet and Dr. B. Schick, *Die Serumkrankheit*, Leipzig, 1905.

Many phases of the subject were studied by Rosenau and Anderson[2] late in 1905 and early in 1906. They studied particularly serum anaphylaxis in the guinea-pig. They showed that a single injection of horse serum was harmless to a normal guinea-pig but that a second injection after a definite interval was usually fatal. This "period of incubation" was found to be about 10 days. The poisonous property was shown to have absolutely no connection with the *antitoxic* property of the serum or with the diphtheria *toxins.*

The poisonous principle in serum was found to be quantitatively specific, death only occurring at the second injection with the homologous serum and never with the heterologous. Hemolysis and precipitin formation were excluded as factors. The poisonous property was unaffected by various chemical, physical, and mechanical influences and by age. Guinea-pigs may be sensitized with 1/1,000,000 c.c. of serum and they remain sensitive for a very long time. They were able to sensitize guinea-pigs by feeding them horse meat or horse serum. They showed further that the offspring of sensitive female guinea-pigs were sensitive to a *first* injection of serum; this being the first instance of the experimental demonstration of the hereditary transmission of a hypersusceptibility or tendency to a disease.

Anderson[3] found that female guinea-pigs could transmit to the same offspring hypersusceptibility to horse serum and immunity to diphtheria toxin. This fact is of great importance in testing antitoxic sera and necessitates care in the selection of breeders for guinea-pigs to be used in serum work.

McClintock and King[4] (1906) gave 10 guinea-pigs from 1/250 to 1 c.c. of horse serum by the stomach and 13 days later 6 c.c. of serum, either subcutaneously or intraperitoneally, without causing symptoms in any of them. They conclude that the sensitizing action of horse serum given by the mouth is not nearly so great as when given subcutaneously or intraperitoneally.

Currie[5] (1907) has studied the effect of repeated injections of horse serum in persons admitted for treatment in the city of Glasgow Fever and Smallpox Hospital at Belvidere. He concludes that it is apparent from the facts detailed by him that repeated injections of horse serum induce symptoms of supersensitation in man, but it is also apparent that the same facts lend no countenance to the suggestion that the death of persons suffering from diphtheria is to be apprehended as the result of repeated injections of antidiphtheric serum.

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[1] R. Otto, "Das Theobald Smithsche Phanomen der Serumueberempfindlichkeit," *v. Leuthold-Gedenkschrift*, I Band, 1905.

[2] *Bull. No. 29*, Hyg. Lab., U. S. Pub. Health and Mar.-Hosp. Serv., Wash , 1906.

[3] *Bull. 30*, Hyg. Lab. U. S. Pub. Health and Mar.-Hosp. Serv., Wash., D. C., 1906.

[4] *Jour. Infect. Dis.*, 1906. 3, p. 700.

[5] *Jour. Hyg.*, 1907, 7, p. 35.

Besredka and Steinhardt[1] (1907) studied with much care certain features of hypersusceptibility to horse serum in guinea-pigs; they note that the French sera are much less toxic than those used by Otto in Frankfurt and the sera used by us. Besredka and Steinhardt had a mortality of about 25 per cent when 5 c.c. of serum was given intraperitoneally at the second injection, whereas death was the rule in our experiments under similar conditions. Most of their work was done with doses of 0.05 to 0.25 c.c. given directly into the brain, which either killed or caused grave symptoms in susceptible guinea-pigs. Besredka and Steinhardt lay stress upon the production of "antianaphylaxis," which we termed "immunity." They found that a single injection of serum given into the peritoneum of a sensitized guinea-pig conferred immunity to a subsequent injection of 0.25 c.c. into the brain; in one case the antianaphylaxis was present one and a half hours after the injection into the abdominal cavity. They were unable to demonstrate any protective properties in various organs of immune guinea-pigs.

Nicolle[2] (1907) found that guinea pigs were not susceptible to the necrotic action induced by repeated injections of horse serum, as is the case in rabbits. He also found that daily injections or "spaced" injections, after the method of Arthus, did not induce a high degree of hypersusceptibility in guinea-pigs.

Besredka[3] (1907) questions whether we should not consider this toxic property of horse serum, in human therapy. He suggests that an antitoxic serum, 0.05 c.c. of which when given into the brain will kill or cause grave symptoms in a sensitive guinea-pig, should be considered as above the average toxicity and ought to be excluded from use in man.

Rosenau and Anderson[4] (1907) in a further contribution to the subject studied particularly the relation of anaphylaxis to immunity. In addition to confirming and extending their work on the specific nature of the phenomenon they made further observations on the relation of various physical influences and chemical substances on the reaction. Among other things they brought out the fact that proteids extracted from the bacterial cells and injected into guinea-pigs produced on the second injection the same train of symptoms as in the case of serum anaphylaxis. It was found that in certain instances the hypersusceptibility produced by these bacterial extracts left the animal immune to the corresponding infection.

Vaughan[5] (1906) advanced the theory that the first injection of the strange proteid is broken up into components, one of which is toxic, but that the animal is not poisoned because this breaking up takes place slowly. The cells, however, learn from this lesson how to break up the complex molecule, so that when more of the strange proteid is introduced at the second injection it is violently rent asunder, quickly liberating large quantities of the toxic principle of the complex molecule.

Vaughan and Wheeler[6] (1907) have elaborated this explanation by further studies upon egg-white and bacterial proteids split into poisonous and non-poisonous portions. These authors believe that when egg-white, or the non-poisonous portion of egg-white, is injected into a fresh animal certain cells of the body are so influenced that they elaborate a new ferment, which, in the form of zymogen, remains in the cell until activated by the second injection, when it is set free and splits up the egg-white in a manner similar to that used by Vaughan in the laboratory. Vaughan and Wheeler believe that the effect induced in the animal is the same as that caused by the poisonous portions of egg-white as they have split it up in the retort.

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[1] *Ann. de l'Inst. Past.*, 1907, 21, p. 117.

[2] *Ann. de l'Inst. Past.*, 1907, 21, p. 128.

[3] *Compt. rend. soc. biol.*, 1907, 62, p. 477.

[4] *Bull. No. 36*, Hyg. Lab., U. S. Pub. Health and Mar.-Hosp. Serv., Wash., 1907.

[5] V. C. Vaughan, *Jour. Amer. Med. Assn.*, 1906, 47, p. 1009.

[6] *Jour. Infect. Dis.*, 1907, 4, p. 476.

Gay and Southard[1] (1907) found in guinea-pigs dying from a second injection of serum and in those which had severe symptoms and were later chloroformed, what they considered characteristic lesions. Considerable hemorrhages, rather definitely localized, are the characteristic gross lesion. The hemorrhages may be in one or several organs, gastric hemorrhages being especially frequent. Microscopically there are in addition to the naked eye hemorrhages minute interstitial and oozing hemorrhages. They also claimed to have found fatty changes in voluntary muscle fiber, heart muscle fibers, and in nerve fibers.

Their explanation of serum anaphylaxis in the guinea-pig is as follows: There is a substance in horse serum (anaphylactin) which is not absorbed by the guinea-pig tissue, is not neutralized, and is eliminated with great slowness from the body. When a guinea-pig is injected with a small amount of horse serum the greater part of its elements are quickly got rid of; the anaphylactin remains and acts as a constant irritant to the body cells, so that their activity for the other elements of horse serum is greatly increased. At the end of two weeks of constant stimulation by the anaphylactin a condition is arrived at where if the cells are suddenly presented with a large amount of horse serum they are overwhelmed in the exercise of their increased assimilating functions, and functional equilibrium is so disturbed that local or general death may occur.

Besredka and Steinhardt[2] (1907) found that guinea-pigs could be put in a state of antianaphylaxis by the injection of horse serum into the brain as well as into the pertioneal cavity. They consider it a phenomenon of the same order as the disintoxication *in vitro* of the tetanized brain by antitetanic serum. They found that guinea-pigs could not be sensitized by intracerebral injection.

They think that their results seem to indicate that the phenomena of anaphylaxis and antianaphylaxis are similar to the precipitating and absorbing actions which govern the relation of colloids among themselves.

Besredka[3] (1907) confirms the work of Rosenau and Anderson that the toxicity of horse serum is destroyed by heating to 100° C.; he further states that it is markedly attenuated by varying degrees of temperature down to 55° or 50° C. He found that injection of calcium chloride the day before prevented the appearance of anaphylactic symptoms.

In a second paper, Otto,[4] in 1907, showed that animals may be sensitized by injecting them with the serum of sensitized animals. He believes that the first injection results in a weakening or disappearance of the portions ("rests") of the antigens which are in the body, and thus an apparent "hypersusceptibility" results. The duration of this hypersusceptibility depends upon the amount of serum injected the first time.

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[1] *Jour. Med. Res.*, May, 1907, 16, p. 143.

[2] *Ann. de l'Inst. Past.*, 1907, 21, p. 384.

[3] *Compt. rend. soc. biol.*, 1907, 62, p. 1053.

[4] *Münch. med. Wchnschr.*, 1907, 55, p 1665.

Richet[1] (1907) gives a general review of the subject of anaphylaxis and also some very interesting work on anaphylaxis produced by a substance obtained from the *mytilus edulis*. He found that the blood of a dog sensitized by this substance when injected into an untreated dog sensitized the animal two days later to an injection of the extract.

He thinks that anaphylaxis is due to the presence of a toxicogenic substance, non-toxic of itself, but producing a poison by reaction with the second injection of the extract. In support of this view he states that *in vitro* a mixture of the serum of a sensitized dog and of the extract is more toxic than the extract alone.

Goodall[2] (1907) gives observations on 90 patients who had received two injections of horse serum; of these 43.4 per cent gave either an immediate or accelerated reaction.

Besredka[3] (1907) concludes that the toxicity of therapeutic sera may be measured by means of intracerebral injections into sensitized guinea-pigs.

Measured in this way, different sera show a wide gamut of toxicity, the fatal dose varying from 1/4 to 1/128 c.c. This toxicity resides in the serum and not in the cellular elements.

The sera of horses living under apparently the same conditions have about the same toxicity; individual variations are rare and of little importance.

The difference in the toxicity of sera appears to be due, in the first place, to their origin; and in the second place, to their age.

Sera are hypertoxic on the day of bleeding, and gradually lose their toxicity. This loss, rapid at first, becomes gradual after the tenth day.

All therapeutic sera should be considered toxic within two months of bleeding.

In a general way, all sera that excite grave anaphylactic phenomena in doses of 1/16 to 1/20 c.c., and *a fortiori*, above this amount, should be considered toxic.

Besredka finally states that the technic and dosage by the intracerebral method is rapid, simple, and not expensive.

SYMPTOMS CAUSED BY THE INJECTION OF HORSE SERUM INTO A SENSITIZED GUINEA-PIG.

Very characteristic symptoms are produced by horse serum, either normal or antitoxic, when injected into a susceptible guinea-pig. The symptoms are apparently the same whether the injection is made subcutaneously or into the peritoneum, or whether normal or antitoxic horse serum is used. In five or ten minutes after injection the pig manifests indications of respiratory embarrassment by scratching at the mouth, coughing, and sometimes by spasmodic, rapid, or irregular breathing; the pig becomes restless and agitated. This stage of exhilaration is soon followed by one of paresis or complete paralysis. The pig is unable to stand or, if it attempts to move, falls upon its side; when taken up it is limp. Spasmodic, jerky, and convulsive movements now supervene.

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[1] *Ann. de l'Inst. Past.*, 1907, 21, p. 497.

[2] *Jour. Hyg.*, 1907, 7, p. 607.

[3] *Ann. de l'Inst. Past.*, 1907, 21, p. 777.

Pigs in this stage with complete paralysis may fully recover, but usually convulsions appear, and are almost invariably a forerunner of death. Symptoms appear about 10 minutes after the injection has been given; occasionally in pigs not very susceptible they are delayed 30 to 45 minutes. Only in one or two instances of the several hundred pigs which we have observed have the symptoms developed after one hour. Pigs developing symptoms as late as this are not very susceptible and do not die. The chain of symptoms is exceedingly characteristic. The symptoms do not always follow in the order given. Death usually occurs within an hour and frequently in less than 30 minutes.

If the second injection be made directly into the brain, the symptoms are manifested with explosive violence, the animal frequently dying within two or three minutes. The same is also true if the second injection be made directly into the circulation.

SENSITIZING PRINCIPLE.

We have advanced the theory that the substance which sensitizes the guinea-pig is perhaps the same as that which later poisons it. Profound chemical changes, perhaps in the central nerve cells, are probably produced by the first injection.

We devoted much time to the isolation of this sensitizing principle, without success.

It is unaffected by the various preservatives used for the preservation of antitoxic serum, by drying, by precipitation with ammonium sulphate or magnesium sulphate, or by admixture with diphtheria or tetanus toxins. Formaldehyd likewise has no influence upon this principle. Serum heated to 60° C. for 30 minutes is as potent in sensitizing animals to a subsequent injection as the unheated serum.

The removal of the spleen or the thyroid from the animal before or after receiving the sensitizing dose apparently has no influence upon the development of anaphylaxis.

Gay and Southard first pointed out that if the blood of a guinea-pig which has received a small sensitizing dose of normal horse serum be drawn, the serum collected, and 1.5 c.c. of this be given to a normal guinea-pig, the normal animal is rendered susceptible to a subsequent injection of horse serum 15 days later. This shows that the sensitizing substance, or *anaphylactin* as it is called, is present in the blood serum of a sensitive animal. It must be present in an exceedingly minute amount, for we have shown that the blood of guinea-pigs receiving only 1/500 c.c. of serum contains this substance several months later, and 1.5 c.c. of the serum of such an animal, when injected into a normal animal, renders it sensitive to a subsequent injection 24 hours later of horse serum.

Guinea-pigs may be sensitized by either large or small amounts of serum. Amounts as great as 10 c.c. have sensitized the animals, while amounts as minute as 1/1,000,000 c.c. did likewise. The optimum sensitizing amount, however appears to be from 1/100 to 1/1,000 c.c.

It has appeared to us in our work that the animals are sometimes rendered slightly more sensitive if given a mixture of toxin-antitoxin used in testing serum. This may be due to the fact that the toxin, which is not completely neutralized by the serum, or which becomes disassociated, may lower the resistance of the animal to some extent. Guinea-pigs are sensitized if the injection be given subcutaneously, intraperitoneally, directly into the heart, into the brain, or if fed by the mouth.

THE TOXIC PRINCIPLE.

At one time we made efforts to isolate the active principle in horse serum which causes the symptoms, but as soon as we realized that the toxic principle present in horse serum exerts its action in quantities so minute as to place it almost in the category of the ferments, we realized how hopeless it would be with present technic to isolate this substance. Nevertheless, we devoted much time and study to the relation of this toxic principle to various chemical, physical, and electrical influences. The practical importance of eliminating this toxic principle from horse serum, or of neutralizing it, is at once evident.

We heated serum for various lengths of time, and found that the toxic property is entirely destroyed by heating to 100° C. for 25 minutes.

The toxic principle is not affected by various chemicals such as calcium chlorid, sodium nitrate, magnesium sulphate, ammonium sulphate, and formaldehyd. It is not affected by various ferments, alkaloids, and similar substances, such as taka diastase, pancreatin, rennin, myrosin, invertin, emulsin, pepsin in acid solution and in alkaline solution, ingluvin, malt, papain, stropin, strychnin, morphin, and caffein.

It is not affected by freezing at 15° F., nor by filtration through porcelain, drying, precipitation and dialysis, and exposure to the X-rays.

We found it interesting to compare the toxic effects upon sensitive animals of untreated antitoxic serum, and the precipitated refined antitoxin; bulk for bulk we found them equally toxic. But as the same number of units can be given in half the bulk there is a manifest advantage in using the precipitated serum, as the rashes and other untoward effects of serum depend to some extent upon the volume of serum administered.

The smallest amount of serum given intraperitoneally that we have found to cause death of a guinea-pig is 0.1 c.c. One hundredth of a c.c., when given directly into the heart, is sufficient to cause the death of the animal, while 0.25 c.c. given into the brain is almost invariably fatal. In most of our work, however, we have used five or six c.c. of serum intraperitoneally, and this seems to be the favorite dose of other workers. Certain symptoms in guinea-pigs caused by a second injection of the serum suggested to us that the action might be due to hemolysis or the formation of precipitins. By a large number of experiments, however, we were able to exclude both hemolysis and the formation of precipitins as factors

THE INFLUENCE OF TIME.

It is necessary that a certain time elapse between the first and second injections of the foreign proteid before the toxic action is manifest. This "period of incubation" is from 10 to 12 days and corresponds suggestively with the period of incubation of the serum disease which von Pirquet and Schick place at 8 to 13 days and with the period of incubation of some of the infectious diseases.

If a guinea-pig be given an injestion of serum and then be injected again any time before the eighth day no ill results follow. In other words, the animal has not had time to enter a state of anaphylaxis to the foreign proteid. If, however, the injection be given after the eighth or tenth day the animal is then in a state of anaphylaxis or hypersusceptibility to the foreign proteid. The shortest interval between the two injections in which we have found death to occur is 12 days. The animal, however, remains susceptible a very long time. The longest period which we have observed is 732 days between the first and the second treatment. We have no doubt, however, that the animal remains susceptible during its entire life.

THE SPECIFIC NATURE OF ANAPHYLAXIS.

From our first studies upon hypercusceptibility we were interested in the question, "Is this reaction specific?"

In our work upon the toxic action of horse serum, we showed that this reaction is quantitatively specific for serums. That is, guinea-pigs sensitized with horse serum are subsequently very susceptible to a second injection of horse serum, but only slightly if at all susceptible to a second injection of the serum of other animals, such as rabbit, cat, dog, hog, sheep, chicken, or man. Conversely, guinea-pigs sensitized with the serum of these other animals are not very sensitive to a second injection of horse serum, whereas, they respond actively to a subsequent injection of the same kind of serum as that used for the first injection.

We have further shown that the specific nature of this phenomenon is more marked when proteid substances of widely different nature are used at the first and second injections. Thus a guinea-pig sensitized with horse serum does not react to a subsequent injection of egg-white, vegetable proteid, or milk. A guinea-pig sensitized with milk does not react to the other proteid substances mentioned.

We have recently succeeded in demonstrating more clearly the specific character of the phenomenon we are studying by proving that guinea-pigs may be in a condition of anaphylaxis to three proteid substances at the same time. For instance, a guinea-pig may be sensitized with egg-white, milk, and horse serum, and subsequently react to a second injection of each one of these substances. The guinea-pig may be sensitized by injecting these strange proteids either at the same time or at different times, in the same place or in different places, or by injecting them separately or mixed. The guinea-pig differentiates each anaphylactic-producing proteid in a perfectly distinct and separate manner. The animal is susceptible to the second injection of each one of the three substances in the same sense that it is susceptible to three separate infectious diseases.

One of our animals reacted sharply to the second injection of milk. This guinea-pig presented the usual symptoms from which it gradually recovered. Five hours after the injection of milk, it was given an injection of egg-white, from which it died. These two reactions in so brief a time seem to accentuate the specific nature of the phenomenon we are studying. It also adds weight to our belief that profound chemical changes probably in the central nervous system, rather than morphologic alterations, explain the essential features of the reaction.

FEEDING EXPERIMENTS.

Guinea-pigs may be sensitized by feeding them meat or serum.

Uhlenhuth[1] found that when rabbits were fed with egg albumen by means of a stomach tube their blood, after a while, developed the power to precipitate egg albumen.

Metalnikoff[2] reports that he obtained immunization by feeding one animal with the blood of another species. He fed horses' blood to white rats and noted that after several weeks the serum of the rats developed decided agglutinative and hemolytic properties.

We found that guinea-pigs could be sensitized by feeding them for some days on horse meat, or dried horse serum, mixed with their food. We did not use a stomach tube, as the possibilities of making slight wounds in the esophageal or gastric mucosa would vitiate the feeding experiments, and we knew from our previous work that very small quantities could readily sensitize the animal to a subsequent injection of serum.

The guinea-pigs that had been fed with horse meat or horse serum, after an interval of at least 14 days, were injected with horse serum and reacted in a characteristic manner.

We also found that guinea-pigs could be sensitized to cattle serum by feeding them with beef. Cooking the meat entirely destroyed its sensitizing properties.

The fact that guinea-pigs may be rendered susceptible by the feeding of strange proteid matter opens an interesting question as to whether sensitive guinea-pigs may also be poisoned by feeding with the same serum given after a proper interval of time. If man can be sensitized in a similar way by the eating of certain proteid substances, may not this throw light upon those interesting and obscure cases in which the eating of fish, sea food, and other articles of diet habitually cause sudden and sometimes serious symptoms?

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[1] *Deut. med. Wchnschr.*, 1900, 26, p. 734

[2] *Centralbl. f. Bakt.*, 1901, 29, p. 531.

HEREDITARY TRANSMISSION.

In the course of our work we had the opportunity to test the susceptibility of the young of susceptible female guinea-pigs and we found that hypersusceptibility to the toxic action of horse serum is transmitted from the mother guinea-pig to her young. This function is solely maternal; the male takes no part whatever in the transmission of these acquired properties. Whether this maternal transmission is hereditary or congenital cannot be definitely stated.

We were able to exclude the milk as a factor in transmitting the hypersusceptibility to the toxic action of horse serum by a series of "exchange" experiments. Exchange experiments consist in at once placing guinea-pigs born of a susceptible mother to nurse with an untreated female, and in exchange, the young of the untreated female are at the same time placed to nurse with the susceptible female. From these exchange experiments we learn that the hypersusceptibility is not transmitted to the young in the milk.

We also learned from our experiments that hypersusceptibility may be transmitted from mother to young, whether the mother is sensitized before or after conception.

If an anaphylactic tendency is transmitted from mother to young in man it may explain the severe reaction and death that occasionally takes place following the first injection of serum.

These results upon the hereditary transmission of the susceptibility to the poisonous action of horse serum in guinea-pigs may throw light upon the well-known inherited tendency to tuberculosis in children born of a tuberculous parent.

There are certain analogies between the action of tuberculosis and horse serum. Both produce a hypersensitiveness and also a certain degree of immunity. Now that we have proved that this hypersensitiveness or anaphylactic action in the case of horse serum may be transmitted hereditarily in guinea-pigs, may it not throw light upon the fact that tuberculosis "runs in families"? While there are several recorded instances demonstrating that immunity to certain infectious diseases may be transmitted from a mother to her young, this is, as far as we know, the first recorded instance in which hypersensitiveness, or a tendency to a disease, has been experimentally shown to be hereditarily transmitted from a mother to her young.

ACTION OF HORSE SERUM UPON MAN AND OTHER ANIMALS.

It may be that man cannot be sensitized in the same way that we have shown to be the case with guinea-pigs. We made no human experiments, but experimental data are recorded by others which have a direct bearing on this question.

Repeated injections of horse serum into man is not an infrequent occurrence. Patients suffering with diphtheria are often given injections of antitoxic serum at short and frequent intervals. It is also not rare for persons to have several attacks of diphtheria at long intervals and to be treated each time with antidiphtheric serum.

Certain sera, for example, the antitubercle serum of Maragliano, or the antirheumatic serum of Menzer, are habitually used by injections at intervals of days or weeks.

In all of these cases of frequent and repeated injections the amount which has been injected and the interval between the injections must be taken into account in relation to this work. Pirquet and Schick in their work on *Serumkrankheit* give eight instances in which children received two injections of horse serum at intervals of from 16 to 42 days between the first and second injections. All these eight cases show this in common, that after the first injection of horse serum, the symptoms of the serum disease appear after the normal period of incubation, namely, between the eighth to thirteenth day. But when the same individuals are again injected with horse serum after intervals of 16 to 42 days, there reappears at once, or at least within 24 hours, symptoms of the serum disease.

Von Pirquet and Schick further give a list of 60 children who were injected with antitoxic horse serum at intervals from six days to seven and a half years between the first and second injections. They found that when the second injection was given from 14 days to four months after the first injection, they obtained, with great regularity, what they termed "the immediate reaction," but when the interval between the first and second injection is over four months they obtained little or no immediate reaction, but what they termed "an accelerated reaction," for the fever, urticaria, and other symptoms of the serum disease appeared on the fifth, sixth, seventh, or eighth day. It will be remembered that the normal period of incubation for the symptoms of the serum disease to appear after the first injection is between the eighth and thirteenth day. Von Pirquet and Schick lay special stress upon the phenomena of the "immediate" and "accelerated" reactions following the second injection.

We might also conclude, despite the suggestion in our work upon sensitizing guinea-pigs by feeding them with horse serum or horse meat, that children are not sensitized to the toxic action in horse serum by eating horse meat from the fact that horse meat is a favorite article of diet in certain European countries, and there is nothing on record to show that the infection of horse serum in those countries is fraught with more danger than where this practice does not obtain. We must, however, remember that our work has shown that guinea-pigs are sensitized with exceedingly minute quantities of the strange proteid, and that repeated injections cause an immunity; and it is possible that the same action may be true of feeding. Further, we have shown that cooking destroys the sensitizing property of meat.

Man reacts to the first injection of horse serum after a period of incubation of 8 to 13 days. Guinea-pigs show practically no reaction following the first injection. Both react to a second injection. The reactions in man and the guinea-pig, however, differ both in severity and kind. The relation, therefore, that our observations upon the guinea-pig may have in its application to man must await further study. Of course, the fact that other animals besides man and guinea-pigs react to a second injection of horse serum would seem to indicate that we are dealing with one and the same action.

We have tested monkeys, rabbits, mice, dogs, cats, rats, chickens, and pigeons to determine whether any of these animals may be sensitized to the action of horse serum. Thus far we have obtained a response in the dogs, rabbits, and cats.

Von Pirquet and Schick also found that the first injection into rabbits caused no clinical effect, but that subsequent subcutaneous injections caused immediate reaction in the production of local edema which extended even to gangrene. Second injections, when introduced intravenously, produced symptoms of collapse and even death.

Arthus also found that the injection of horse serum into rabbits caused no symptoms, whether the horse serum was injected subcutaneously or intravenously, but when he injected the serum every six days subcutaneously, he obtained, after the fourth injection, edema and local reaction which continued to gangrene.

A rabbit which von Pirquet and Schick had previously treated with eight injections was then given 2 c.c. of horse serum into the ear vein. The rabbit reacted to this in one minute and, after characteristic symptoms similar to those we see in guinea-pigs, died in about four minutes.

Arthus obtained similar results with repeated injections of sterilized milk.

IMMUNITY TO ANAPHYLAXIS.

We showed in our first publication on the subject of anaphylaxis that guinea-pigs may be *actively* immunized against this phenomenon. At the same time we demonstrated that the immunity could not be transferred *passively* to other animals in the blood serum or body juices.

Guinea-pigs may be actively immunized in several ways: (1) by repeated injections of serum during the period of incubation: that is, during the first 10 days, before the animal reaches the state of hypersusceptibility; (2) by recovery from a second injection given during the anaphylactic stage.

The fact that guinea-pigs cannot be immunized passively by the transference of blood or body juices would make it appear that the "immune body," if such exists against the toxic action of horse serum, is not free in the blood or body juices, as is the case in diphtheria. In fact it has been questioned whether the active immunity which we have described is an instance of true immunity, or a "refractory" condition, or even an actual return to the normal.

For instance, Besredka and Steinhardt speak of immunity to anaphylaxis as "antianaphylaxis." They state that this immunity is not analogous to that conferred by the specific sera against certain bacteria and their toxins; also that the intraperitoneal injection of a serum is able to protect a sensitized guinea-pig against a cerebral injection given a few hours later is never seen in the case of the specific sera. Besredka and Steinhardt state that the immunity conferred by the specific bacterial antisera is very transient; it lasts generally a few weeks at the most. On the other hand they find that in the case of antianaphylaxis to the serum phenomenon, the time is much longer, viz., many months. They ask the question, Is not this immunity to anaphylaxis simply due to the return of the sensitized guinea-pig to a normal state—in other words, a process of desensitization?

Gay and Southard also doubt immunity to anaphylaxis and consider such guinea-pigs to be rather in a "refractory" condition due to a prolongation of the period of incubation in the case of a large initial dose, or of multiple doses, in proportion to the amount of serum injected.

Subsequent researches have strangthened our belief that we are dealing with a true condition of immunity and not a prolongation of the period of incubation or a return to the normal. Thus it has been shown that guinea-pigs in the "refractory" condition still contain anaphylactin in their blood. It is at once evident that they have not returned to their normal condition. Further, we have demonstrated that such "refractory" female guinea-pigs will transmit this anaphylactic substance to their young. Only the sensitizing substance passes into the blood of the foetus, which is therefore in a condition of hypersusceptibility. The "immunizing substance" or "condition" is not hereditary or congenital.

It seems to us that we have here a striking analogy to that phase or kind of immunity which von Pirquet describes as "allergie." In other words, we have an acquired immunity associated with anaphylaxis. In guinea-pigs this immunity may follow one attack of the disease, i. e., the serum reaction. As stated by von Pirquet, allergie manifests itself by an immediate reaction and corresponds to the condition of immunity conferred by an attack of smallpox or of some of the other acute infectious processes.

In the case of syphilis we have a striking instance in which the vrus is not auto-inoculable. In the serum reaction in the guinea-pig an analogous train of events occurs, for after the sensitized guinea-pig has responded, the reaction renders the organism immune. A somewhat similar immunity to hypersusceptibility is shown in the tuberculin reaction. Thus Vallée[1] has recently shown in studying the cutaneous reaction of tuberculous cattle, goats, and guinea-pigs that if tuberculous cattle be given a subcutaneous injection of tuberculin a few days before the cutaneous test is applied the latter reaction is prevented. Further, Calmette, Breton, and Petet[2] have found that rabbits free from tuberculosis, if given a small dose of tuberculin in the ear vein and then tested 16 hours later for the ophthalmic reaction to tuberculin, show a typical reaction; if the same rabbits are tested 48 hours later in the other eye the result is negative or very feeble, thus showing that an immunity to the reaction is acquired by the first reaction. This seems to be another example of allergie, or immunity to a specific hypersusceptibility.

HYPERSUSCEPTIBILITY AND IMMUNITY PRODUCED BY BACTERIAL PROTEIDS.

We believe that the problem of hypersusceptibility has an important bearing upon the question of immunity, and hence we expressed the opinion that "resistance to disease may largely be gained through a process of hypersusceptibility. Whether this increased susceptibility is an essential element or only one stage in the process of resistance to disease, must now engage our attention." We cannot escape the conviction that this phenomenon of hypersusceptibility has an important bearing on the prevention and cure of certain infectious processes. Our work upon the hypersusceptibility produced by the bacterial proteids strengthens this belief, for our recent results prove that the phenomenon of hypersusceptibility to certain proteid substances extracted from the bacterial cell is followed by a definite immunity against infection by the micro-organism.

Experimental studies with the bacterial proteids are of the greatest importance on account of the practical uses to which results along this line may lead.

Hypersusceptibility may easily be induced in guinea-pigs with proteid extracts obtained from the bacterial cell. The first injection of most of the extracts used by us seems comparatively harmless to the animal. A second injection of the same extract shows, however, that profound physiological changes have taken place. A definite period must elapse between the first and the second injection. The symptoms presented by the guinea-pigs as a result of the second injection resemble those caused by horse serum.

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[1] *Med. Vet.*, 84, p. 308.

[2] *Compt. rend. de soc. biol.*, 1907, 63, p. 296

The phenomenon induced by a second injection is followed (in certain cases) by an immunity to the corresponding infection. The following is a summary of our experimental results along these lines.

Extract of colon bacillus.—The extract from the colon bacillus used in our experiments was obtained as follows:

A two-day-old culture of *B. coli communis* in Dunham's solution was used to heavily inoculate the surface of 84 large agar plates. These plates were grown at 37° C. for four days and the surface growth collected.

The bacterial mass was frozen 48 hours at about 15° F., thawed at room temperature, and then ground with sand by hand in a mortar for five hours, shaken vigorously half an hour, and again frozen 18 hours. After again thawing, the fluid was diluted with salt solution and filtered through a Berkefeld filter. The clear filtrate gave a distinct coagulum with heat and acetic acid.

All the other extracts were obtained by a similar process. In the case of the tubercle bacillus, the bacterial mass was first washed three days in running water to eliminate the soluble tuberculin as much as possible.

The hypersusceptibility induced by the colon extracts manifested itself by symptoms resembling those already described in the case of horse serum. The guinea-pigs scratched at the mouth with their hind legs. Most of them showed evidences of respiratory embarrassment by quickened, labored, or irregular breathing. Many of the pigs lay over on their sides, a common symptom. A few developed jerky movements; but in no case was convulsion noted. The pigs looked quite sick and ill at ease, but gradually recovered, so that by next morning they seemed normal.

Ten days following the second injection of the extract the guinea-pigs were given 5 c.c. of a heavy emulsion of colon bacillus from 24-hour-old agar slants, but showed no symptoms, and remained in good condition. Three controls received the same injection and died in twelve hours.

Anthrax.—A number of the guinea-pigs were given the extract from anthrax bacilli before infection; some were given a single injection, some two injections, and others daily injections for 20 days. Other guinea-pigs were given the extract used as a vaccine, both in single and repeated injections, after being infected with anthrax Bacilli. The extract did not seem to have any influence on the course of the disease, whether given before or after the infection.

Tuberculosis.—The guinea-pigs which have reacted to two injections of proteid extract obtained from the tubercle bacillus are now being tested for immunity to infection with tubercle cultures.

Typhoid.—The indications of hypersusceptibility induced by two injections of typhoid extract manifest themselves by rapid respirations; most of the pigs lie down on their sides. The symptoms presented by this series of pigs were mild.

Nine days following the second injection of the extract five pigs, which had received

10 c.c. of the typhoid extract at the second injection, resisted a large dose of a virulent typhoid culture. Two controls died in 18 hours. One or two of the pigs which had received the extract were slightly sick the following day, but the next day had fully recovered and have remained so. A definite immunity was, therefore, conferred by the two injections of extract from the typhoid bacillus.

These results strengthen our belief that the phenomenon of hypersusceptibility has a practical significance in the prevention and cure of certain infectious processes. It gives a possible explanation to the period of incubation of some of the communicable diseases. Is it a coincidence that the period of incubation of a number of infectious diseases is about 10 to 14 days, which corresponds significantly with the time required to sensitive animals with a strange proteid? In certain infectious diseases with short periods of incubation, such as pneumonia, the crisis which commonly appears about the tenth day may find a somewhat similar explanation. It is evident that disease processes produced by soluble toxins, such as diphtheria and tetanus, do not belong to the category now under consideration.

The phenomenon of hypersusceptibility has been produced in the guinea-pig by extracts obtained from the colon bacillus, yeast, hay bacillus, anthrax, tubercle bacillus, and the typhoid bacillus. The hypersusceptibility produced by the colon and typhoid bacillus was followed by a definite immunity to the corresponding infection. In the case of anthrax, however, immunity does not follow hypersusceptibility to the anthrax proteid. We are, therefore, not dealing with a general law applicable to all infections, but with certain limitations as in the case of antitoxic immunity.